

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Withdrawn) An isolated oligonucleotide comprising at least one ditag, wherein the ditag includes two joined first and second sequence tags, and wherein the first tag includes a 5'-terminus sequence and a second tag comprises the 3'-terminus sequence of a nucleic acid molecule or fragment thereof.
2. (Withdrawn) The oligonucleotide of claim 1, further comprising two adapters flanking the ditag, wherein each adapter includes at least one restriction site.
3. (Withdrawn) The oligonucleotide of claim 2, wherein each adapter comprises at least a first restriction site which is an asymmetric restriction site and at least a second restriction site.
4. (Withdrawn) The oligonucleotide of claim 3, wherein the asymmetric restriction site comprises a type II restriction site.
5. (Withdrawn) The oligonucleotide of claim 1, wherein the nucleic acid molecule comprises the full-length sequence of a gene or a fragment thereof.
6. (Withdrawn) The oligonucleotide of claim 1, wherein the nucleic acid comprises RNA, mRNA, genomic DNA, full-length cDNA, or cDNA.
7. (Withdrawn) The oligonucleotide of claim 1, wherein the ditag is obtained by splicing the 5'terminus and the 3'terminus of the nucleic acid molecule or fragment

thereof in the presence of at least one restriction enzyme and the size of the sequence tags is determined by the restriction enzyme used.

8. (Withdrawn) The oligonucleotide of claim 7, wherein the restriction enzyme is a type II restriction enzyme.

9. (Withdrawn) The oligonucleotide of claim 7, wherein the restriction enzyme is selected from the group consisting of AarI, AceII, Alol, BaeI, Bbr7I, BbvI, BbvII, BccI, Bce83I, BceAI, BceFI, Bcgl, BciVI, Bfil, BinI, BplI, BsaXI, BscAI, BseMII, BseRI, BsgI, BsmI, BsmAI, BsmFI, Bsp24I, BspCNI, BspMI, BsrI, BsrDI, BstF5I, BtgZI, BtsI, CjeI, CjePI, EciI, Eco3II, Eco57I, Eco57MI, Esp3I, Fall, Faul, FokI, GsuI, HaeIV, HgaI, Hin41, HphI, HpyAv, Ksp632I, MboII, MlyI, MmeI, MnlI, PleI, PpiI, PstI, RleAI, SapI, SfaNI, SspD5I, Sth132I, StsI, TaqII, TspDTI, TspGWI, TspRI, TthIII, I-CeuI, PI-SceI, PI-PspI and I-SceI.

10. (Withdrawn) The oligonucleotide of claim 1, wherein the ditag size is 12-60 bp.

11. (Withdrawn) The oligonucleotide of claim 1, wherein the ditag comprises 34-38 nucleotides and the size of each tag is determined by the use of restriction enzyme MmeI.

12. (Withdrawn) The oligonucleotide of claim 1, wherein the first and second tag have the same or a different number of nucleotides.

13. (Withdrawn) The oligonucleotide of claim 1, wherein the oligonucleotide consists of 1 to 1000 ditags.

14. (Withdrawn) A vector comprising an isolated oligonucleotide including at least one ditag, wherein the ditag includes two joined first and second sequence tags, and wherein the first tag includes a 5'-terminus sequence and a second tag comprises the 3'-terminus sequence of a nucleic acid molecule or fragment thereof.

15. (Withdrawn) The vector of Claim 14 further comprising two adapters flanking the ditag, wherein each adapter includes at least one restriction site and wherein each adapter includes at least a first restriction site which is an asymmetric restriction site and at least a second restriction site.

16. (Withdrawn) The vector of claim 15, wherein the backbone of the vector does not comprise the asymmetric restriction site or the second restriction site.

17. (Withdrawn) The vector of claim 16, wherein the asymmetric restriction site is a type II restriction site.

18. (Withdrawn) A vector comprising at least a nucleic acid molecule and two adapters flanking the nucleic acid molecule, wherein each adapter comprises at least: a first restriction site which is a type II restriction site and at least a second restriction site, and wherein the backbone of the vector does not comprise the type II restriction site, or the second restriction site.

19. (Withdrawn) The vector of claim 18, wherein the type II restriction site is Mmel.

20. (Withdrawn) A vector comprising SEQ ID NO:18.

21. (Withdrawn) The vector of claim 20, comprising at least one ditag, wherein the ditag includes two joined first and second sequence tags, and wherein the first tag

includes a 5'-terminus sequence and a second tag comprises the 3'-terminus sequence of a nucleic acid molecule or fragment thereof.

22. (Withdrawn) The vector of claim 21, comprising two adapters flanking the ditag, wherein each adapter includes at least one restriction site and wherein each adapter includes at least a first restriction site which is an asymmetric restriction site and at least a second restriction site.

23. (Withdrawn) A cDNA library, wherein every cDNA clone of the library comprises at least two oligonucleotide including at least one ditag, wherein the ditag includes two joined first and second sequence tags, and wherein the first tag includes a 5'-terminus sequence and a second tag comprises the 3'-terminus sequence of a nucleic acid molecule or fragment thereof.

24. (Withdrawn) The cDNA library of claim 23, wherein the at least one oligonucleotide comprises 1-1000 ditags.

25. (Currently Amended) A method for preparing at least one ditag comprising the steps of:

(i) producing at least one full-length ~~coding sequence of a~~ cDNA transcript, said transcript having a 5' terminus including the 5' transcription start site and a 3' terminus including the 3' transcription endpoint;

(ii) cleaving the full-length coding sequence of the cDNA transcript at its 5' terminus to extract a 5' tag having a 5' end and a 3' end and including the 5' transcription start site and cleaving the full-length coding sequence of the cDNA transcript at its 3' terminus to extract a 3' tag having a 5' end and a 3' end and including the 3' transcription endpoint; and

(iii) generating at least one ditag by ligating the 3' end of the 5' tag to the 5' end of the 3' tag; wherein the at least one ditag comprises a 5'-terminus sequence and a 3'-terminus sequence of ~~[[a]] the full-length coding sequence of a gene~~ cDNA transcript.

26. (Currently Amended) A method for preparing at least one ditag comprising the steps of:

(i) providing at least one full-length ~~coding sequence of a~~ cDNA transcript having a 5' terminus including the 5' transcription start site and a 3' terminus including the 3' transcription endpoint and flanked by two adapters;

(ii) cleaving the full-length ~~coding sequence of the~~ cDNA transcript at its 5' terminus to extract a 5' tag having a 5' end flanked by an adapter and a 3' end and cleaving the full-length coding sequence of the cDNA transcript at its 3' terminus to extract a 3' tag having a 5' end and a 3' end flanked by an adapter; and

(iii) generating at least one ditag flanked by two adapters by ligating the 3' end of the 5' tag to the 5' end of the 3' tag; wherein the at least one ditag comprises a 5'-terminus sequence including the 5' transcription start site and a 3'-terminus sequence including the 3' transcription endpoint of a full-length coding sequence of a gene the full-length cDNA transcript.

27. (Previously Presented) The method of claim 26, further comprising the step of creating a concatemer of ditags.

28. (Cancelled)

29. (Previously Presented) The method of claim 26, further comprising including the at least one ditag flanked by the adapters in a vector.

30. (Cancelled)

31. (Currently Amended) The method of claim 26, further comprising the step of determining the nucleotide sequence of the at least one ditag ~~to detect gene expression.~~

32. (Currently Amended) The method of claim 26, further comprising:
determining the sequence of the at least one ditag; and
mapping the 5' and 3' tags of the at least one ditag nucleotide sequence to a database comprising genomic sequences.

33. (Currently Amended) The method of claim 26, wherein each adapter comprises at least one restriction site and step (ii)
includes adding at least one restriction enzyme capable of recognizing the at least one restriction site to the full-length ~~coding sequence of the~~ cDNA transcript flanked by the two adapters from step (i).

34. (Previously Presented) The method of claim 33, wherein the at least one restriction site is an asymmetric recognition site.

35. (Previously Presented) The method of claim 34, wherein the asymmetric recognition site is a restriction endonuclease asymmetric cleavage site sequence recognizable by a type II restriction enzyme elected from the group consisting of AarI, AceIII, AclI, BaeI, Bbr7I, BbvI, BbvII, BccI, Bce83I, BceAI, Bcefl, Bcgl, BciVI, Bfil, BinI, BpII, BsaXI, BscAI, BseMII, BseRI, BsgI, BsmI, BsmAI, BsmFI, Bsp24I, BspCNI, BspMI, BsrI, BsrDI, BstF5I, BtgZI, BtsI, CjeI, CjePI, EciI, Eco3II, Eco57I, Eco57MI, Esp3I, Fall, Faul, FokI, GsuI, HaeIV, HgaI, Hin4I, HphI, HpyAV, Ksp632I, MboII, MlyI, MmeI, MnlI, PleI, PpiI, PstI, RleAI, SapI, SfaNI, SspD5I, Sth132I, StsI, TaqII, TspDTI, TspGWI, TspRI and Tth111II.

36. (Previously Presented) The method of claim 34, wherein the asymmetric recognition site is a homing endonuclease asymmetric recognition site sequence recognizable by a homing endonuclease selected from the group consisting of: I-CeuI, PI-SceI, PI-PspI and I-SceI.

37. (Currently Amended) The method of claim 26 wherein each adapter comprises at least one restriction site which is a MmeI recognition site; and step (ii) comprises

cleaving the full-length ~~coding sequence of the~~ cDNA transcript flanked by the two adapters from step (i) with MmeI.

38. (Previously presented) The method of claim 26, wherein the at least one ditag comprises 34-38 nucleotides.

39. (Currently Amended) A method for genome mapping, comprising:
preparing at least one ditag according to the method of claim 25, and the ditag
~~including~~

~~two joined first and second sequence tags, wherein the first tag includes the 5'-terminus sequence and the second tag includes the 3'-terminus sequence of a full-length coding sequence of a cDNA transcript, corresponding to the full-length coding region of a gene;~~

mapping ~~each of the 5' and 3' two~~ tags of the at least one ditag on the genome sequence; and

defining the entire structural region of the corresponding gene on the genome map, wherein the region being defined includes exons and introns of the gene.

40. (Currently Amended) A method of gene discovery comprising:
preparing at least one ditag according to the method of claim 25, the ditag
~~including two joined first and second sequence tags, wherein the first tag includes the~~

~~5'-terminus sequence and the second tag includes the 3'-terminus sequence of a full-length coding sequence of a cDNA transcript, the cDNA transcript corresponding to the full-length coding region of a gene;~~

determining the sequence of the at least one ditag and

comparing the sequence of the at least one ditag with a genome sequence

[[map]] and a gene database;

wherein detecting matching of the sequence of the 5' and 3' termini tags of the at least one ditag [[on]] with the genome [[map]] sequence but detecting no match [[on]] with one or more gene databases indicates a new gene.

41. (Currently Amended) The method of claim 40, further comprising recovering a full-length cDNA corresponding to the at least one ditag by PCR or directly from target RNA samples by RT-PCR.

42. (Withdrawn) A method for recovering full-length cDNA comprising:

preparing, from a full-length cDNA library, at least one oligonucleotide including at least one ditag, the ditag including two joined first and second sequence tags, wherein the first tag includes the 5'-terminus sequence and the second tag includes the 3'-terminus sequence of a full-length cDNA;

sequencing the obtained oligonucleotide ditag;

determining the ditag of interest; and

recovering the full-length cDNA corresponding to the ditag of interest from the full-length cDNA library.

43. (Withdrawn) A method for quantifying the transcriptional activity of a gene comprising:

preparing, from a full-length cDNA library, at least one oligonucleotide comprising at least one ditag, the ditag including two joined first and second sequence tags,

wherein the first tag includes the 5'-terminus sequence and the second tag includes the 3'-terminus sequence of a full-length cDNA;
sequencing the obtained oligonucleotide ditag;
determining the frequency of the sequenced ditag which corresponds to the transcriptional activity of the gene.

44. (Currently Amended) The method of claim 29, wherein the vector comprises at least one ditag, wherein the at least one ditag includes two joined first and second sequence tags, and wherein the first tag comprises the 5'-terminus sequence and a second tag comprises the 3'-terminus sequence of the full-length coding sequence of the cDNA transcript.

45. (Currently Amended) The method of claim 44, wherein the vector further comprises two adapters flanking the at least one ditag, wherein each adapter includes at least one restriction site.

46. (Previously Presented) The method of claim 53, wherein the backbone of the vector does not comprise the asymmetric restriction site or the second restriction site.

47. (Previously Presented) The method of claim 46, wherein the asymmetric restriction site is a type II restriction site.

48. (Previously Presented) The method of claim 29, wherein each adapter comprises at least: a first restriction site which is a type II restriction site and at least a second restriction site, and wherein the backbone of the vector does not comprise the type II restriction site or the second restriction site.

49. (Currently Amended) The method of claim 48, wherein the type II restriction site is a Mmel site.

50. (Previously presented) The method of claim 29, wherein the vector comprises SEQ ID No:18.

51. (Canceled)

52. (Canceled)

53. (Previously presented) The method of claim 45, wherein each adapter comprises at least a first restriction site which is an asymmetric restriction site and a second restriction site.

54. (Canceled)